Appendix A

Microbial Analysis of Subsurface Disposal Area Extraction Well Samples

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INTRODUCTION

Site and Operation Description

The Radioactive Waste Management Complex (RWMC) is on the southwest portion of the Idaho National Engineering and Environmental Laboratory (INEEL). The facility encompasses three major operational areas: the Subsurface Disposal Area (SDA), the Transuranic Storage Area, and a combined operations and administration area. Since 1952, transuranic and low-level radioactive waste has been buried in pits, trenches, and soil vaults and stored on aboveground pads. The waste is reported to contain other nonradioactive hazardous materials, such as mercury, beryllium, asbestos, zirconium fines, solidified acids and bases, solvents and degreasing agents, and sodium and potassium salts, in addition to radionuclides. Since burial, a large amount of radioactive and nonradioactive material has been released to the surficial soil and the vadose zone directly beneath the SDA. Contaminants of interest to the current study are the volatile organic compounds detected in the SDA-area soil gas. These volatile organic compounds (VOCs) include acetone; benzene; 2-butanone; carbon tetrachloride; chloroform; methylene chloride; tetrachloroethene; toluene; 1,1,1-trichloroethane; and trichloroethene (Miller and Navratil 1998). The primary contaminants of interest are carbon tetrachloride, chloroform, and trichloroethene. Carbon tetrachloride contamination extends vertically from land surface to the water table and laterally greater than 1 km (0.62 mi) from the SDA boundary. Concentrations from 3,000 to 5,000 ppmv have been measured in the center of the SDA above the B-C interbed at 33.5 m (110 ft). Concentrations increase with depth down to the B-C interbed and then decrease down to the C-D interbed at 73 m (240 ft).

Perched water has been detected in six wells within the RWMC above the B-C and C-D interbeds. Thirty-two contaminants have been identified in the perched water; 14 of the contaminants were found at concentrations above the maximum contaminant levels for the Site. The VOCs listed above, as well as nitrate, have been observed in perched water and lysimeter samples taken since 1988.[a] Perched water also represents an operational problem in that it can infiltrate vadose zone wells and interfere with monitoring and normal operation.

Under the *Federal Facility Agreement and Consent Order* (DOE-ID 1991), organic contamination in the vadose zone (OCVZ) was identified for an RI/FS (Duncan, Troutman, and Sondrup 1993) as Operable Unit (OU) 7-08. Completion of the RI/FS eventually led to the Record of Decision (ROD), *Declaration for Organic Contamination in the Vadose Zone Operable Unit 7-08*, finalized in November 1994 and signed in December 1994 (DOE-ID 1994). Operable Unit 7-08 deals specifically with the OCVZ from land surface to the top of the Snake River Plain Aquifer, approximately 177 m (580 ft) below land surface located outside the disposal pits and trenches of the SDA. The vadose zone contains VOCs that are primarily in the form of organic vapors, which have migrated from the buried waste in the pits at the SDA. The selected remedy, as identified in the ROD (DOE-ID 1994), was extraction and destruction of organic contaminant vapors in the vadose zone accompanied by monitoring in the vadose zone and in the underlying aquifer. The general objective of the selected remedy is to reduce the risks to human health and the environment associated with the organic contaminants present in the vadose zone and to prevent

a. Brady Lee, Personal Communication with Kirk Dooley, "Perched Water Contamination," May 2000.

federal and state drinking water standards from being exceeded after the 100-year institutional control period. The primary remedial action objective for the OCVZ Project, as identified in the ROD (DOE-ID 1994), is to ensure that risks to future groundwater users are within acceptable guidelines and that future contaminant concentrations in the aquifer remain below federal and state maximum contaminant levels.

The major components of the selected remedy include the following:

- Installation and operation of five vapor extraction wells (in addition to an existing vapor extraction well) at the RWMC as part of a first phase effort to extract organic contaminant vapors from the vadose zone. The selected remedy includes options to expand the number of vapor extraction wells for potential second and third phases.

- The installation and operation of off-gas treatment systems to destroy the organic contaminants present in the vapor removed by the extraction wells. Off-gas treatment will be in the form of catalytic oxidation or an equally effective organic contaminant destruction technology.

- The addition of soil-vapor monitoring wells to monitor the performance of the vapor extraction wells and verify the attainment of remedial action objectives.

Three vapor vacuum extraction units with recuperative flameless thermal oxidation treatment units were designed, built, and installed within the boundaries of the SDA to meet the objectives of the ROD (DOE-ID 1994). The recuperative flameless thermal oxidation treatment unit designated Unit A was initially designed to extract from vapor extraction Wells 8901D and 4E but currently treats vapors from Well 8901D because Well 4E is plugged. Unit B normally extracts from extraction Wells 2E and 3E, but 3E is currently not being used and is shut down because it is plugged. In 2001, the third recuperative flameless thermal oxidation treatment unit was replaced with a catalytic oxidizer designated as Unit D. Unit D currently extracts from Well 7V.

Over the operating period from January 1996 through June 2001, the vapor vacuum extraction process has removed and treated approximately 27,006 kg (59,538 lb) of carbon tetrachloride; 6,304 kg (13,898 lb) of chloroform; 5,885 kg (12,974 lb) of trichloroethene; 1,638 kg (3,611 lb) of 1,1,1-trichloroethane; and 1,390 kg (3,064 lb) of tetrachloroethene. Over this operating period, the treatment units operated for approximately 70% of the available time. During processing downtime, VOC concentrations were allowed to rebound because active airflow through the wells was shut down.

Definition of Problem

Fouling or plugging of extraction wells represents a significant problem in the optimum operation of a vapor vacuum extraction system. As indicated above, at least two wells have become plugged over a relatively short time period during the operation of the various units used to remediate the vadose zone at the RWMC. Typical extraction wells at the RWMC (See Figure A-1) consist of a 10-in. well bore containing a 4-in. polyvinyl chloride pipe screened at the interval where the vapors will be extracted from the subsurface. The annular space of the well is then completed by surrounding the screened interval with coarse sand or gravel, which is then covered with bentonite clay. The bentonite clay is allowed to expand after the addition of water. Grout or concrete is then used to finish and seal the screened interval. Completed wells are then plumbed to an extraction unit for contaminant removal from the vadose zone.

As Wells 3E and 4E were operated, air permeability decreased eventually leading to plugging of the well, making the wells unsuitable for use. Video logs of the well casings from an additional well (3V) indicated a grayish brown viscous material oozing from the slots in the screened interval of well, an indication that microbial activity may be in part responsible for the plugging of the wells.

Scope of Research

A number of microbial enrichment and physiological tests were proposed to determine whether the plugging of the wells was caused by the growth of microorganisms in the gravel or sand pack in the annular space of the screened interval. Approximately 10 to 15 g of a gray-brown material was collected from the internal surface of the well bore from Well 3V. The sample contained not only the grayish brown material but also polyvinyl chloride filings from the screened interval where the sample was taken.

Subsamples of the material were used to perform tests to determine enrichments for iron-reducing bacteria (because iron bacteria are common in biofouling in water wells) and total heterotrophs to estimate the total number of heterotrophs in the sample. Tests were also performed to determine the effect of temperature on growth; the various sources of ferric iron on growth; and, since the samples were taken from an area that at one point was exposed to carbon tetrachloride and other chlorinated VOCs, the ability of the culture to reductively dechlorinate carbon tetrachloride.

EXPERIMENTAL METHODS

Microbial Enrichment

Subsamples of the material from the well casing were added to approximately 50 mL of M1 growth medium for enrichment of facultative iron-reducing bacteria (Kostka and Nealson 1998). The growth medium was buffered with 10 mM HEPES, and 20 mM lactate was used as the electron donor. Soluble Fe(III) (10 mM) was supplied as Fe(III) EDTA. To establish anaerobic conditions for enrichment, the M1 medium was dispensed into 160 mL serum vials, which were stoppered with sterile styrofoam plugs and stored overnight in an anaerobic chamber with a headspace of 4% hydrogen and 10% carbon dioxide in nitrogen bulk gas. Following equilibration, approximately 0.5 g (0.02 oz) of well material was added, and the vials were aseptically capped using butyl rubber septa and aluminum crimp rings. Cultures were then incubated at room temperature and monitored for growth.

Following the primary enrichment from the well casing material in M1 medium, a secondary enrichment was performed in which aliquots of growing cultures were inoculated into fresh M1 growth medium. Secondary enrichment was performed using aerobic and anaerobic starting conditions. Aerobic enrichments were performed using M1 growth medium that had not been stored in the anaerobic chamber. Since iron-reducing bacteria are facultative anaerobes, aerobic conditions were used to allow dense growth prior to the microbes switching their metabolism to use Fe(III) as the terminal electron acceptor.

Numbers of total heterotrophic microorganisms were determined by adding 5 g of the material from the well casing to 25 mL of phosphate buffer. After blending in a sterile stainless steel blender head, the sample was serially diluted to 10^{-4}. Sample sizes of 0.1 mL of the serial dilutions (10^{-3} and 10^{-4}) were then spread onto agar plates containing trypticase soy agar, incubated at room temperature and monitored for growth.

Effect of Fe(III) Source on Growth

Using cells from one of the aerobically-started iron-reducing enrichments, an experiment was set up to determine the effect of material used to construct the well on growth of the culture. Well construction material used included quartz sand, crushed basalt, and bentonite clay. These materials represent sources of insoluble Fe(III) and may act as electron acceptors for growth for the microbes of interest. Approximately 0.1 g (0.004 oz) of each material was used in each assay vial. Three treatments were set up for each material: one set containing the insoluble iron source and inoculum, one set with the

insoluble iron source with no inoculum, and a final set with the insoluble iron source that had been sterilized by autoclaving. A vial of M1 growth medium containing Fe(III) EDTA will be used as a soluble iron control. These control vials were set up with and without inoculum.

Each assay was set up under aerobic conditions and was incubated statically at a temperature of 15°C. Growth was monitored by determining lactate utilization, oxygen utilization, and ferrous iron production. Lactate and other organic acids were monitored using high-pressure liquid chromatography. Oxygen utilization was monitored using gas chromatography, and ferrous iron production was monitored using the ferrozine dye assay.

Similar testing was performed using vials started under anaerobic conditions.

Dechlorination Assays

An initial experiment was set up to determine whether the microbial consortia enriched from the well-casing material could reductively dechlorinate carbon tetrachloride. The soluble iron control experiments described above were used for testing. Vials were decapped and then amended with 20 mM lactate and 5 mM Fe(III) EDTA. The vials were then capped, and carbon tetrachloride was added to a final concentration of 12 mg/L. Uninoculated controls were set up in a similar manner. A vial containing M1 growth medium without iron was used as a blank to monitor abiotic disappearance of carbon tetrachloride. Cultures were incubated statically at 15°C. Carbon tetrachloride concentrations were monitored using capillary gas chromatography.

A second set of experiments was set up to determine the effect of various sources of ferric iron on reductive dechlorination of carbon tetrachloride by the SDA enrichment culture. Sources of ferric iron included Fe(III) EDTA (5mM), basalt, and silica sand (10 g/150 mL growth medium). Amber vials (250 mL) containing 150 mL of M1 growth medium and 20 mM lactate were used for the dechlorination experiments. A target carbon tetrachloride concentration of 10 mg/L was used for testing. Uninoculated controls were set up for each source of ferric iron tested as well as a blank containing no cells or source of ferric iron. All constituents but the carbon tetrachloride were added to the growth medium, the vials were inoculated with the well enrichment, and then were capped with mininert valves. After a 30-minute equilibration period, the first sample was taken.

Analytical

Oxygen

Determination of oxygen in the cultures was performed using a Hewlett-Packard 5890 Series II Gas Chromatograph controlled with HP 3365 Chemstation software. Concentrations were determined using a thermal conductivity detector operated at 225°C. Peaks were separated using a 25-m Chrompack Plot fused silica column with a 0.53 mm internal diameter, containing a 50 μm film of Molsieve 5Å as the stationary phase. Helium was used as the carrier gas. The injector was held at 200°C, and a sample size of 200 μL was used for analysis.

Organic Acids

Lactate, acetate, propionate, butyrate, and formate concentrations were monitored using high-pressure liquid chromatography. Samples were prepared by adding 100 μL of sample to 800 μL of distilled water in a microcentrifuge tube. The samples were then acidified using 4N H_2SO_4 for a final dilution of 1:10 for the samples. After 10 minutes of incubation at room temperature, the samples were

filtered through a 0.2-μm nylon syringe filter (Gelman Sciences). Filtered samples were frozen until the analysis was performed.

The high-pressure liquid chromatography was equipped with a Hitachi L-4000H UV detector set at a wavelength of 208 nm. Samples were eluted using 0.05 N H_2SO_4 pumped using an L-6200A Intelligent Pump at a flow rate of 0.375 mL min^{-1} and a pressure of 770 psi. The volatile fatty acids were separated using a Perkin Elmer Brownlee, Polypore H, 220 x 4.6-mm column, packed with 10 μm of sulfonated 8% cross-linked polystyrene cation exchange resin in the hydrogen form. Automated sampling was performed using a Hitachi AS-4000 Intelligent Autosampler and sample size of 20 μL.

Ferrous Iron

Iron concentration was determined spectrophotometrically using the ferrozine dye method (Kostka and Nealson 1998). Samples were extracted into 0.5M hydrochloric acid and frozen before analysis. The extract was then added to a 0.02% ferrozine buffered with 50 mM HEPES. The solution was then incubated for 15 minutes, filtered through a 0.2-μm nylon syringe filter, and then measured on the spectrophotometer at a wavelength of 562 nm. Iron measurements were standardized using ferrous ammonium sulfate over a concentration range from 0.01 to 5 mM.

Carbon Tetrachloride

Concentrations of carbon tetrachloride were measured by gas chromatography using a Hewlett-Packard 5890 Series II Gas Chromatograph. Operation of the gas chromatograph and data collection were accomplished using Hewlett-Packard 3365 Chemstation software. When analyzing carbon tetrachloride, the gas chromatograph was equipped with a Restek 30 m, 0.32-mm internal diameter, and Rtx-5 column containing a 0.25-μm film thickness of crossbonded 95% dimethyl-5% diphenyl polysiloxane (Restek Corp, Bellefont, Pennsylvania). A flame ionization detector held at 275°C, using nitrogen as the makeup gas, with an injector temperature of 250°C was used to analyze carbon tetrachloride. Runs were isothermal at 100°C.

RESULTS AND DISCUSSION

Primary Enrichment

Aliquots of the grayish brown sample material from the well casing of Well 3V were added to M1 growth medium, which had been stored under anoxic conditions. M1 growth medium is an enrichment medium for the cultivation of iron-reducing bacteria. The medium was amended with 20 mM lactate as the carbon and energy source and 10 mM ferric iron as Fe(III) EDTA. Lactate was used because it is a common carbon source for the cultivation of iron-reducing bacteria while a soluble form of ferric iron was used to increase the probability of growth.

Growth medium in the four enrichment vials was yellow upon initiation of the experiment. After incubating for 7 to 9 days, growth medium in the vials began to clear, indicating that iron reduction was occurring. Light clouds that may have been bacteria began to form on the bottom of the vial. After 13 days of incubation, the enrichment vial that showed clearing in 7 days showed the presence of a gelatinous substance on the bottom of the vial. The substance is some sort of unidentified extracellular material that is likely to be an extracellular polysaccharide produced by microbes in the culture. Visual analysis of the culture indicated that this substance absorbed all solid material in the culture including bacteria, the initial solid material from the inoculation and polyvinyl chloride filings in the sample. One other enrichment vial showed the formation of ribbons of material within the growth medium, which eventually formed a gelatinous mass as discussed above. While the formation of the gelatinous substance

was demonstrated in vials for the enrichment of iron-reducing bacteria, it is not known whether the iron reducers are responsible for the formation of the extracellular polysaccharide.

Approximately 3 mL of the culture from the primary enrichment were transferred to fresh growth medium. Two vials that had been stored under anaerobic conditions and another set stored under toxic conditions were inoculated. This experiment was run to determine whether the iron-reducing population could grow in the presence of oxygen, which is possible since some iron reducers are facultative anaerobes. In this case, facultative cultures will grow initially using oxygen as a terminal electron acceptor and then switch to iron as the terminal electron acceptor following depletion of the oxygen. After two days of incubation, the culture started under aerobic conditions was showing substantial growth while the anaerobic start was showing minimal growth. Within approximately 1 week, the culture started aerobically showed iron reduction. Cultures that started using anaerobic media took approximately 2 weeks before iron reduction was noted by visual examination.

Secondary enrichments of the original culture isolated from material taken from extraction Well 3V appear to be depleted in their ability to produce extracellular material. Production of extracellular material is evident in the cultures, but the extent of congealing seen in the original enrichment culture has not been noted. One potential cause for this lack of congealing is that inorganic matter in the original well sample was required for maximum extracellular material production and congealing.

Trypticase soy agar was used to determine total heterotrophs in the sample. After 2 days of growth, approximately 1.3×10^6 microbial cells for each gram were seen of the substance scraped from the well bore. Following 5 days of incubation at room temperature, the volume of cells increased to 2.4×10^7 microbial cells for each gram of substance. These types of cell densities are common for a rich soil but should be considered unusually high for the surface of polyvinyl chloride pipe.

Effect of Fe(III) Source on Growth

Experiments were run to determine whether the microbial population isolated from the well casing material could reduce iron found in material used to construct the well. Insoluble sources of ferric iron were silica sand used as packing in the screened interval; bentonite clay, which is used to seal the well; and crushed basalt chips as drill cuttings obtained as waste from the well-drilling process. Lactate and oxygen removal, as well as ferrous iron production, were compared during growth of the microbial population from the well. These experiments could also indicate whether these materials are potential sources of microbes for the fouling of the wells.

Figure A-2a shows the lactate removal and acetate production from vials containing silica sand while Figure A-2b shows oxygen removal and ferrous iron production. Vials receiving the inoculum from the well culture were able to remove lactate until oxygen removal leveled off. Though the graph indicates that approximately 20% of the initial oxygen was present, it is believed that the instrument may have reached the lower level of detection and that oxygen levels are closer to zero. A small amount of Fe(II) was generated during the experiment. A small amount of Fe(II) was transferred during inoculation, but there was an additional increase during testing, indicating the microbes were able to use insoluble Fe(III) found on the silica sand. After Day 8, the microbial population began to remove the acetate. Slow gradual removal of lactate and oxygen was noted in uninoculated vials, but no Fe(II) production was noted. Vials containing silica sand that were not inoculated showed no lactate or oxygen removal.

Test results for the bentonite clay can be seen in Figures A-3a and A-3b. Lactate and oxygen utilization occurred immediately in the inoculated cultures. Lactate removal in these vials leveled off until Day 8, followed by nearly complete removal by the end of the experiment. The production of Fe(II) increased to nearly 1 mM during the test period, showing the highest production of the insoluble Fe(III)

sources tested. By the end of the testing period, there was a large amount of broken up polymer and the biomass. The polymer developed a dark gray tint indicating the presence of sulfate-reducing bacteria. Acetate levels in the inoculated vial leveled off between days 8 and 15. The uninoculated control showed slower lactate and oxygen removal than the inoculated vials. A small amount of Fe(II) was produced initially, but after oxygen was removed, the amount of Fe(II) increased to near 0.5 mM. No lactate or oxygen removal or Fe(II) production was noted in the sterile controls. These results indicate that the bentonite is a source of iron-reducing microbes as well as microbially reducible iron for the well-casing microbial population.

Visual examination of the growth medium from the vials containing bentonite as the insoluble Fe(III) source showed the development of a cloudy material at the bottom of the vial similar to that seen during enrichment of the material from the well casing. After approximately 1 week, a large amount of polymer-like material began to develop. By the end of the experiment, the material began to break up as noted with the previous enrichment experiments.

Tests results to determine the effect of basalt as a source of microbial reducible iron can be seen in Figures A-4a and A-4b. Vials receiving the inoculum enriched from the well casing demonstrated some lactate removal, which leveled off after 5 days. Oxygen was completely removed within 7 days, and a small amount of Fe(II) was produced. Acetate concentration reached a maximum on Day 5 and decreased to near zero indicating that the microbes may be preferentially using the acetate rather than the lactate. Vials receiving no inoculum and the sterile controls showed no lactate removal and only a small amount of oxygen removal in the uninoculated vials.

Figures A-5a and A-5b show test results from the vials containing soluble Fe(III) in the form of Fe(III) EDTA. As with the silica sand and the crushed lava, lactate and oxygen removal was immediate, but lactate removal leveled off after 5 days. Acetate concentration in the growth medium increased for the first 8 days of the experiment followed by a decrease between days 8 and 15. Production of Fe(II) increased to above 2 mM and then leveled off. Polymer production was noted in the vials receiving inoculum. Solid material in the vials became light gray at the end of the experiment indicating the potential presence of sulfate-reducing bacteria. Though a small amount of Fe(II) was produced in uninoculated controls, no lactate or oxygen removal was noted indicating that the iron reduction may have been abiotic.

From the results generated, it appears that all three well construction materials are sources of microbially reducible iron as well as microbes that could potentially cause fouling of the well. It is not understood why the silica sand, basalt, and M1 grown enrichments only removed a portion of the lactate and then stopped. One potential explanation is that the microbes used all of the electron acceptor present (i.e., oxygen initially followed by Fe[III]) so they stopped growing, the microbes preferentially degraded the acetate produced, or community members that degraded acetate became dominant after 8 days. The presence of the dark gray material in the bentonite vials indicated the presence of iron sulfide produced by sulfate-reducing bacteria. The presence of sulfate and subsequent reduction may account for the continued removal of lactate in these cultures.

Reductive Dechlorination of Carbon Tetrachloride

Since the well from which the test culture was enriched was exposed to carbon tetrachloride, experiments were conducted to determine whether the culture could reductively dechlorinate carbon tetrachloride. Three assays were conducted to monitor dechlorination: one that was inoculated with the enrichment culture, one containing growth medium with no inoculum, and a control containing distilled water.

Preliminary results for carbon tetrachloride removal from the various assays can be seen in Figure A-6. After 16 days of incubation, the carbon tetrachloride concentration in the inoculated vials decreased from approximately 12 ppmv of carbon tetrachloride to near 4 ppmv. Carbon tetrachloride removal was initiated after the oxygen had been removed from the vials. Oxygen removal was greater than 70% after the second day of incubation. Similar results were demonstrated for the iron reducer *Shewanella putrefaciens* 200, which demonstrated higher rates of carbon tetrachloride reduction and chloroform production when grown microaerophilically (Picardal et al. 1993). Small peaks attributed to products of dechlorination were noted on the gas chromatograph chromatograms after Day 8 but were not identified or quantified. These peaks were not seen in the uninoculated and control vials. Minimal carbon tetrachloride removal was noted in the uninoculated and control vials.

Carbon tetrachloride concentrations decreased in all of the vials used for testing (see Figure A-7). The maximum decrease was noted in vials containing Fe(III) EDTA and crushed basalt. Reductive dechlorination in these vials was verified by appearance and a substantial increase in chloroform concentrations in the headspace of the vials (see Figure A-8). Maximum chloroform production was seen in the Fe(III) EDTA vials (>10 mg/L) while approximately 6 mg/L was produced in vials containing crushed basalt. Small amounts of chloroform appeared in the sand and the crushed basalt control, but levels were well below 1 mg/L. No chloroform was detected in the other controls and the blank vials.

The ability of the culture to dechlorinate carbon tetrachloride is not surprising since numerous strains of iron-reducing bacteria have demonstrated the ability to reductively dechlorinate compounds such as carbon tetrachloride (Picardal, Arnold, and Huey 1995; Backhus et al. 1997), 1,2-dichloroethene, and vinyl chloride (Bradley and Chapelle 1997).

SUMMARY AND CONCLUSIONS

Plugging of vacuum vapor extraction wells within the SDA at the RWMC (a waste storage site at the INEEL) has called for the determination of potential agents that may have led to the fouling of the wells. One potential mechanism for plugging of the wells would be the growth of microbes in the sand and gravel pack within the annular space surrounding the screened interval for the extraction wells. Experiments were performed to determine microbial numbers and the presence of iron-reducing bacteria from material obtained from the inner wall of the well casing near the screened interval of the well. In addition, testing was performed to determine whether material used to construct the well could be used as a source of Fe(III) for growth of the microbes. Finally, since the well had been exposed to carbon tetrachloride during operation, tests were performed to determine whether the culture enriched from the well casing material could reductively dechlorinate carbon tetrachloride.

Initial enrichments of the well material showed the presence of a high density of microbes as well as the presence of iron-reducing bacteria, which were a subpopulation of a fairly diverse microbial population. In addition to iron reduction, the initial enrichment appeared to demonstrate the production of an extracellular polymer that absorbed all solid material within the culture medium. Secondary enrichment from these cultures into aerobic and anaerobic media yielded heavy growth in the aerobic

starts followed by iron reduction and slow growth in the anaerobic starts. Extracellular polymer production in the secondary enrichments was depleted compared to the primary enrichment.

Silica sand, bentonite clay, and crushed lava rock were tested as potential sources of microbially reducible iron as well as a source of microbes for the well plugging. All three well construction materials could apparently be used as sources of microbially reducible iron as indicated by the production of Fe(II). The most interesting results were generated from the test vials containing bentonite clay. A cloudy layer developed in the test vials, which was similar to that seen in the vials used for the primary enrichment. In addition, the largest amount of extracellular polymer was produced in these vials compared to the other insoluble iron sources as well as the vials containing Fe(III) EDTA. The bentonite also acted as a source of iron-reducing bacteria.

Preliminary research results also indicated that the enrichment culture was able to reductively dechlorinate carbon tetrachloride when Fe(III) EDTA was present as the electron acceptor. Additional microcosms were set up to determine whether the culture could reductively dechlorinate carbon tetrachloride using insoluble sources of ferric iron. Cultures were started under aerobic conditions, and when most of the oxygen had been removed, reductive dechlorination commenced. In addition to reductive dechlorination using Fe(III) EDTA, the culture produced chloroform when crushed basalt was used as a source of ferric iron. Liquid samples were taken from the cultures during growth and were frozen for DNA extraction, which will occur at a later date. Additional research needs to be performed to determine the effect of oxygen and additional electron donors including hydrogen. Additional research into iron cycling and the effect of nitrate concentrations on reductive dechlorination needs to be performed.

The current reductive dechlorination experiments were performed under saturated conditions. Additional research needs to be performed to determine the ability of the culture to reductively dechlorinate carbon tetrachloride under unsaturated conditions. Most of the carbon tetrachloride beneath the SDA is contained within the vadose zone so the ability of the culture to dechlorinate under unsaturated conditions is important.

From the data generated in the research discussed above as extraction well operation, the following potential mechanism for well fouling has been generated. At various points during operation, part or all of the silica sand gravel pack may have become saturated as the result of the infiltration of water from the surface or condensation from humidity in the air stream. Once the packing was saturated, microbes from the well construction material or the geological media surrounding the well began to grow in the liquid within the packing, producing extracellular material, which partially plugged the packing. Some of the water saturating the packing may have evaporated, but the areas that had become infiltrated with microbes and polymer would have caused the airflow to go through the paths of least resistance. As similar saturation events occurred over time, the well eventually became plugged.

Observations from the primary enrichment of the well material, as well as the tests with the bentonite, indicate that the bentonite may have become dissolved in the water and aided in the formation of the extracellular polymer. Microbes present may have acted to enhance the thickening properties of bentonite by producing an extracellular slime, which polymerized with the bentonite to form the viscous material noted. This observation may also explain why there was less polymer production in secondary enrichments. Since the amount of the material from the bentonite would have been diluted or absent, material required for stability of the extracellular polymer was not present so there was no or little polymer formed.

Once plugging has occurred, wells must be remediated at relatively high expense. Remediation options include removal of the screened interval to remove plugging material, flooding of the well with a

hypochlorite or mildly acidic solutions to dissolve the plugging material, or least desirable, filling the well with concrete and drilling a new extraction well. Installation of preventive measures during well construction may be feasible and could represent a fairly cost-effective method of controlling fouling of extraction wells. Preventive measures could include some type of antimicrobial compound, such as powdered or granular hypochlorite, which will inhibit microbial growth in the packing material. When water infiltrated the packing, these antimicrobials would be released, inhibiting growth of microbes present.

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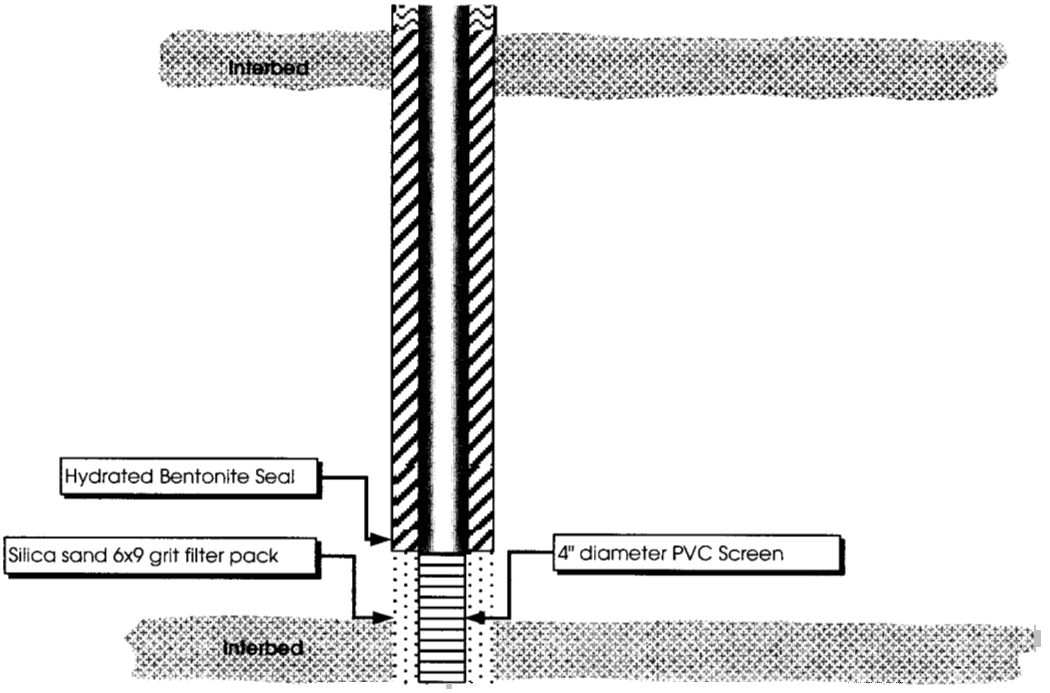

Figure A-1. Schematic of conceptual vadose zone vapor extraction well showing screened interval of well.

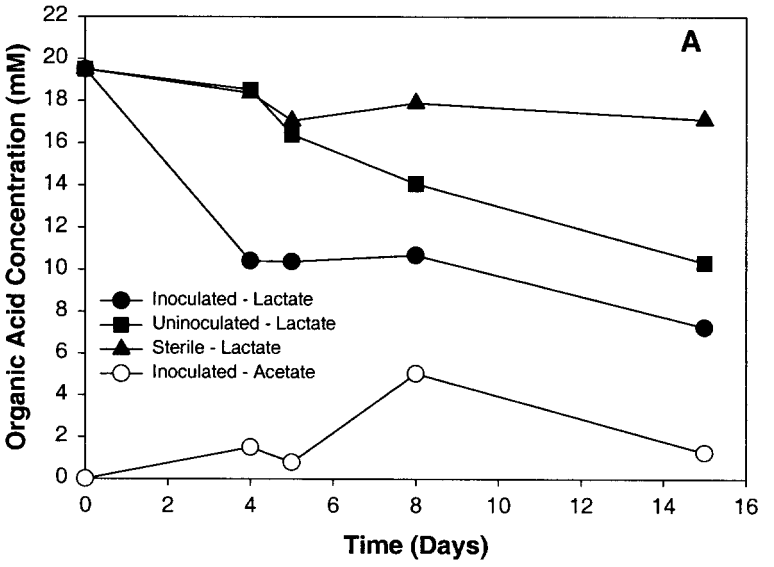

Figure A-2a. Lactate removal and acetate production in test vials containing silica sand as the source of insoluble Fe(III).

Note: Closed symbols represent lactate concentration; open symbols represent acetate concentration. No acetate was noted in uninoculated and sterile control so the data has not been added to the graph.

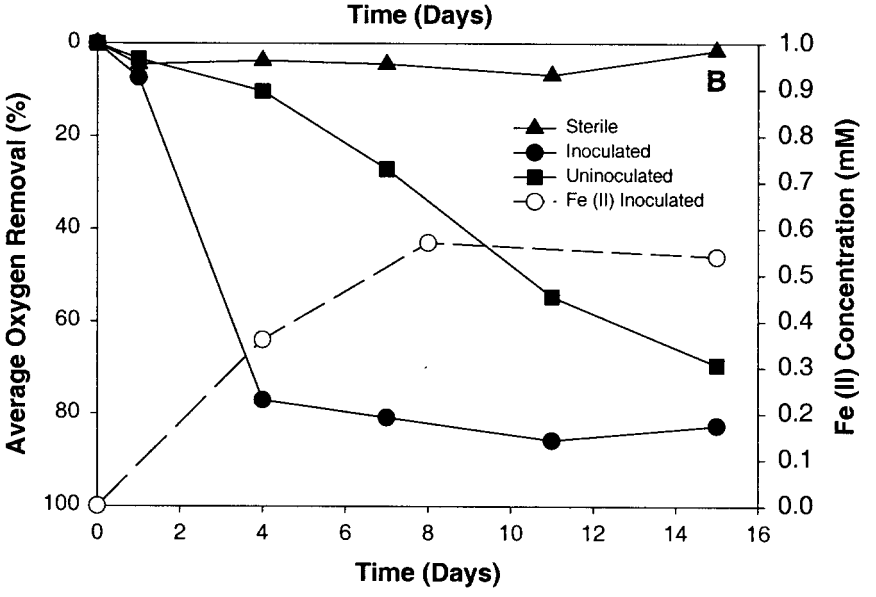

Figure A-2b. Oxygen removal and Fe(II) production in test vials containing silica sand as the source of insoluble Fe(II).

Note: Closed symbols represent oxygen removal while open symbols represent ferrous iron production. No ferrous iron was produced in the sterile controls or uninoculated vials so the data has been left off of the graph.

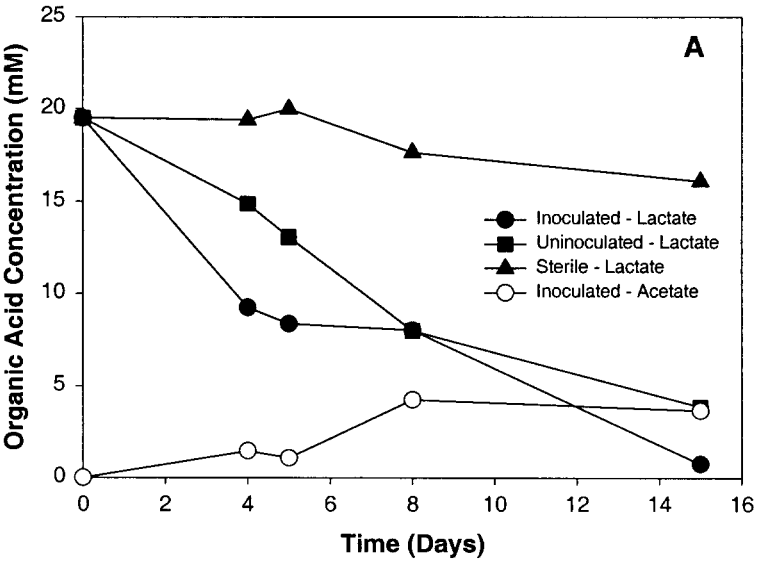

Figure A-3a. Lactate removal and acetate production in test vials containing bentonite clay as the source of insoluble Fe(III).

Note: Closed symbols represent lactate concentration; open symbols represent acetate concentration. No acetate was noted in uninoculated and sterile control so the data has not been added to the graph.

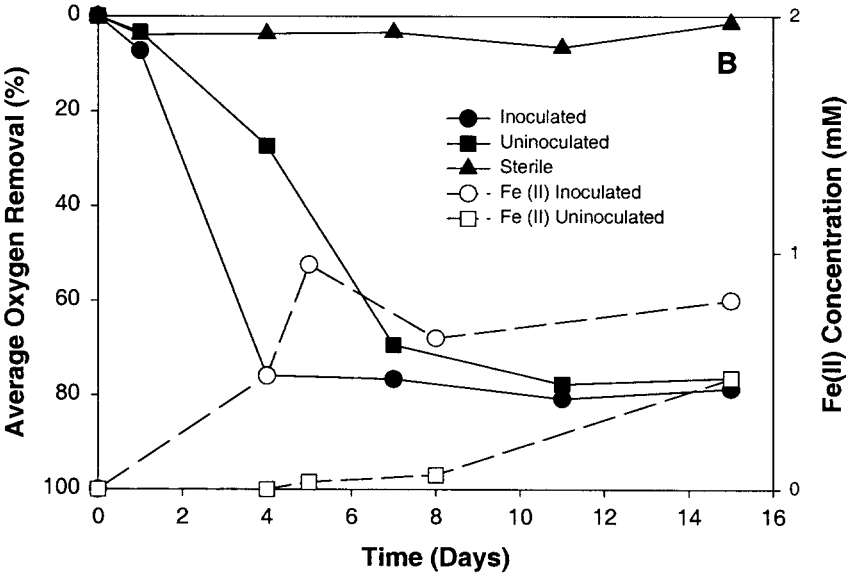

Figure A-3b. Oxygen removal and Fe(II) production in test vials containing bentonite clay as the source of insoluble Fe(II).

Note: Closed symbols represent oxygen removal while open symbols represent ferrous iron production. No ferrous iron was produced in the sterile control so the data has been omitted.

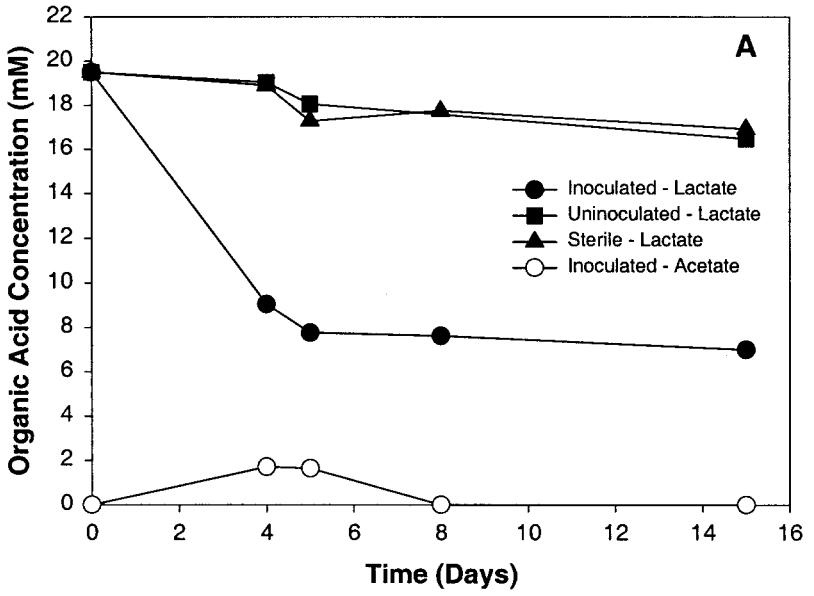

Figure A-4a. Lactate removal and acetate production in test vials containing crushed basalt as the source of insoluble Fe(III).

Note: Closed symbols represent lactate concentration; open symbols represent acetate concentration. No acetate was noted in uninoculated and sterile control so the data has not been added to the graph.

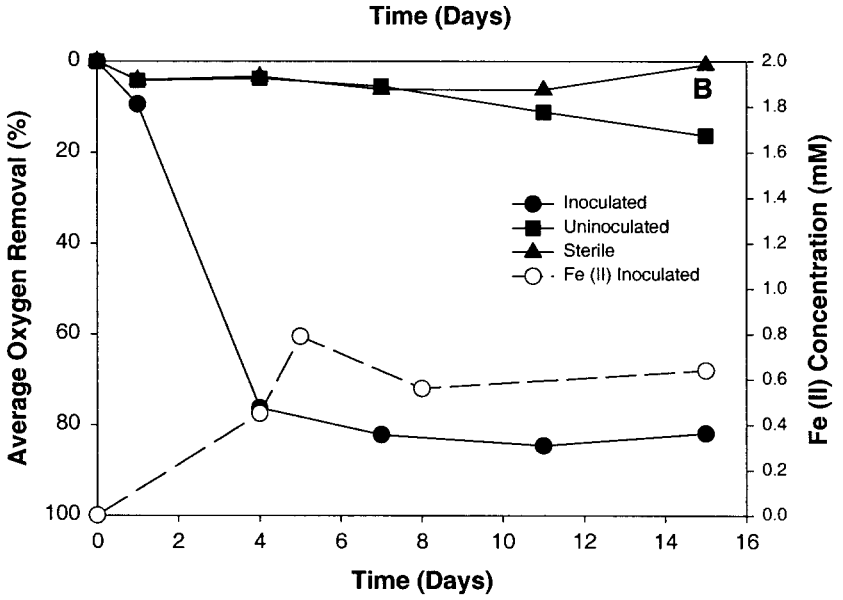

Figure A-4b. Oxygen removal and Fe(II) production in test vials containing crushed basalt as the source of insoluble Fe(II).

Note: Closed symbols represent oxygen removal while open symbols represent ferrous iron production. No ferrous iron was produced in the sterile controls or uninoculated vials so the data has been left off of the graph.

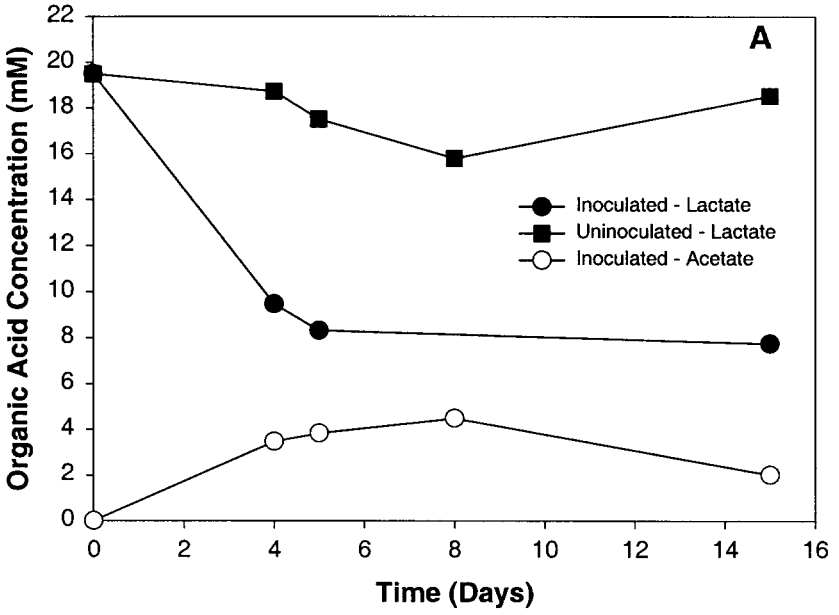

Figure A-5a. Lactate removal and acetate production in test vials containing Fe(III) EDTA as the source of soluble Fe(III).

Note: Closed symbols represent lactate concentration; open symbols represent acetate concentration. No acetate was noted in uninoculated and sterile control so the data has not been added to the graph.

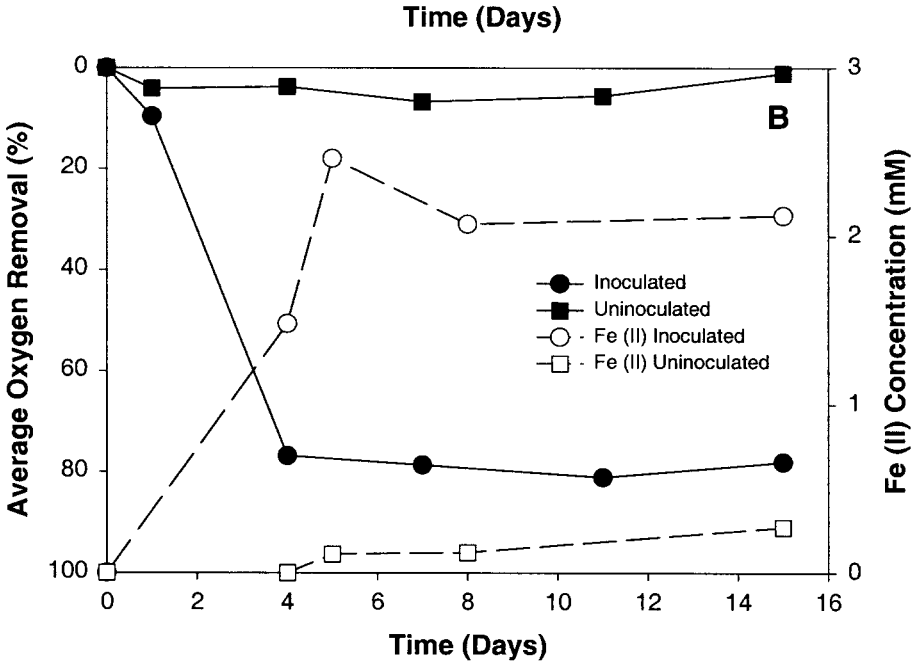

Figure A-5b. Oxygen removal and Fe(II) production in test vials containing Fe(III) EDTA as the source of soluble Fe(II).

Note: Closed symbols represent oxygen removal while open symbols represent ferrous iron production.

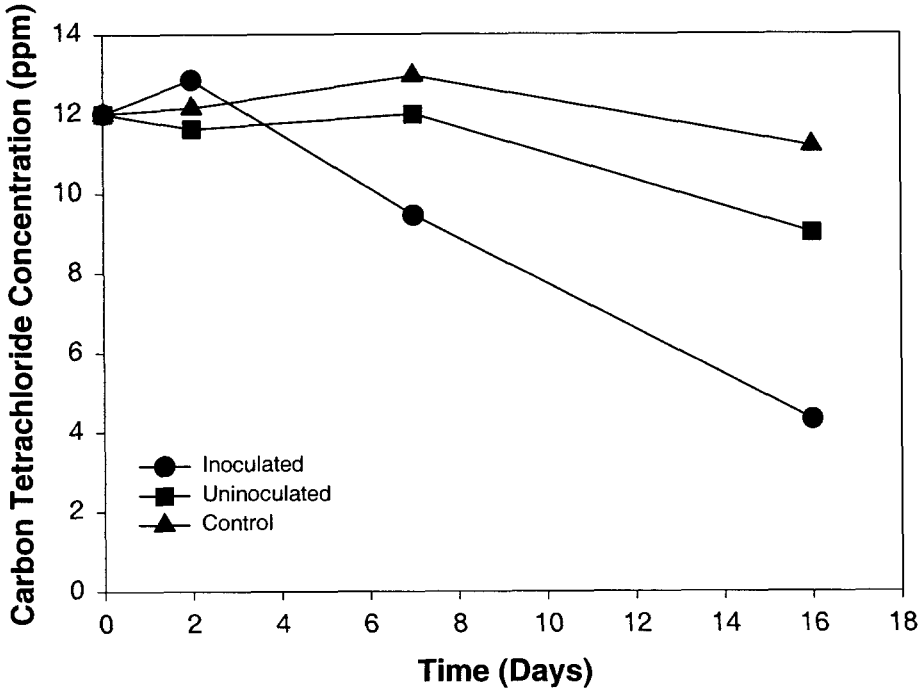

Figure A-6. Carbon tetrachloride removal by microorganisms enriched from material taken from the internal wall of vapor extraction Well 3V.

Note: Tests were initiated under aerobic conditions. Fe(III) EDTA was used as an alternate electron acceptor following depletion of oxygen.

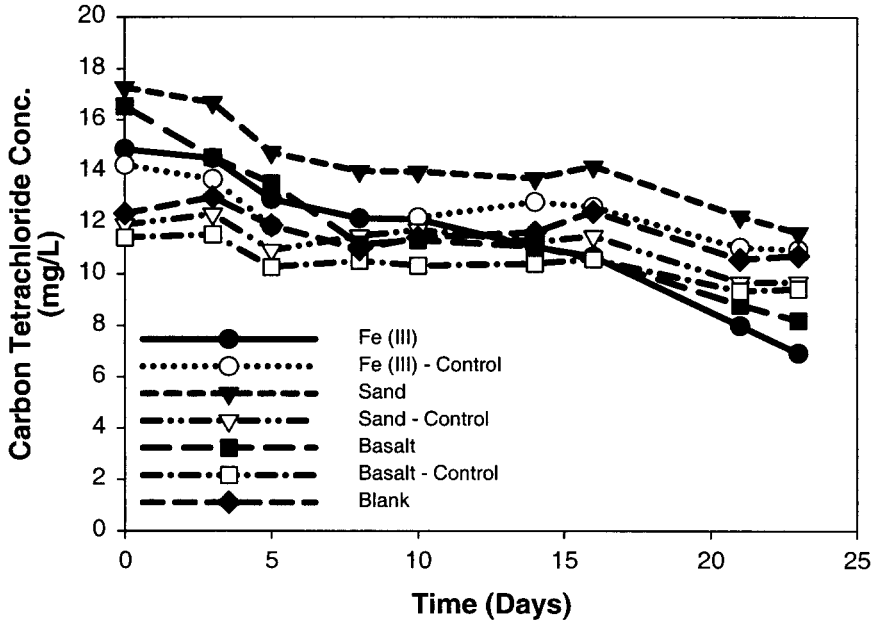

Figure A-7. Carbon tetrachloride removal by Subsurface Disposal Area culture in the presence of various soluble and insoluble sources of ferric iron.

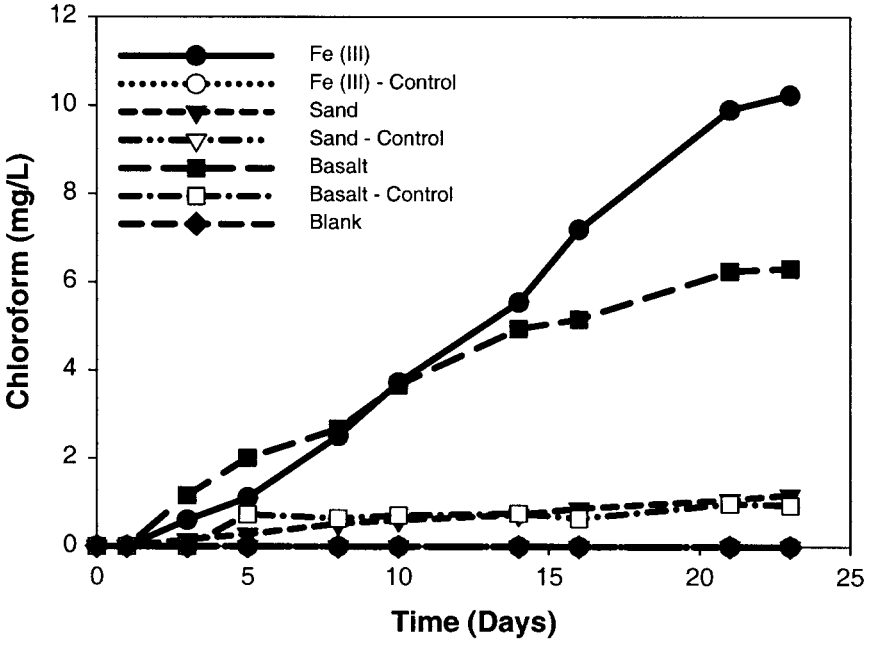

Figure A-8. Chloroform production by Subsurface Disposal Area culture in the presence of various soluble and insoluble sources of ferric iron.

Appendix B

Prefinal Inspection Documentation

Appendix B

Prefinal Inspection Documentation

This appendix contains the Prefinal Inspection Checklist used by the U.S. Department of Energy, the Idaho Division of Environmental Quality, and the U.S. Environmental Protection Agency to authorize the startup of operation of Vapor Vacuum Extract with Treatment Unit D. A copy of the letter indicating completion of the inspection is included.

Y. Harvego 3920

Department of Energy
Idaho Operations Office
850 Energy Drive
Idaho Falls, Idaho 83401-1563

April 17, 2002

Mr. Wayne Pierre, Team Leader
Environmental Cleanup Office
U.S. Environmental Protection Agency
Region X
1200 Sixth Avenue, Seattle, Washington 98101

Mr. Dean Nygard, Site Remediation Manager
Idaho Department of Environmental Quality
1410 N. Hilton
Boise, Idaho 83706

SUBJECT: Completion of OU 7-08 Unit D Shakedown Period and Transmittal of Completed Unit D Pre-final Inspection Checklist (EM-ER-02-059)

Dear Mr. Pierre and Mr. Nygard:

I am pleased to report that the Vapor Vacuum Extraction with Treatment Unit D operational shakedown period officially ended on March 4, 2002. During the shakedown period initial operating problems with system components and procedures were identified and corrected. Also, the completed Unit D Pre-Final Inspection Checklist is enclosed. Please contact me at (208) 526-4392 or Mark Shaw at (208) 526-6442 with any questions.

Sincerely,

RMShaw for

Kathleen E. Hain, Manager
Environmental Restoration Program

Enclosure

cc: D. Koch, DEQ, 1410 N. Hilton, Boise, ID 83706
M. Wilkening, EPA Region X, Idaho Ops Office, 1435 N. Orchard St., Boise, ID 83706

UNIT D KING BUCK THERMAL CATALYTIC OXIDIZER PREFINAL INSPECTION CHECKLIST

	PROJECT TITLE				
ITEM NO.	ITEM DESCRIPTION	STATUS	DATE	PERSON RESPONSIBLE	COMMENTS
1	PROJECT DOCUMENTS				
a	Review Field Sampling Plan (INEEL/EXT-99-00907, Rev 0, July 2000), Health and Safety Plan (INEL-96/0119, Rev 5, May 2001), and Operations and Maintenance Plan (INEEL/EXT-01-00016, Rev. B. July 2001) to ensure that differences between the King Buck Catalytic oxidizer and the Thermatrix oxidizer are appropriately documented	Complete	9/12/01		FSP includes reference TPR 1662.
b	Operations and Maintenance Plan revision is approved and issued. (INEEL/EXT-01-00016, Rev. B. July 2001)	Complete	8/9/01	Lisa Harvego	
c	The Field Sampling Plan for Operations and Monitoring Sampling Conducted in Support of the Organic Contamination in the Vadose Zone Remediation Project Plan (INEEL/EXT-99-00907, Rev. 0, July 2000) is approved and issued	Complete	9/12/01	Lisa Harvego	FSP includes reference TPR 1662.
d	Waste Management Plan for operations is documented in the O&M Plan (INEEL/EXT-01-00016, Rev. B. July 2001)	Complete	7/17/01		WMP has been incorporated into the O&M Plan.
e	Essential As-Built Drawings completed (P&Ids: 515640, 515641, 51564, electrical: 515650)	Complete	7/17/01		
f	Industrial Hygiene Exposure Assessment completed	Complete	3/12/02	Lisa Harvego	IH sampling and analysis at Unit D coordinated with sampling at Units A and B. Results received 3/12/02.
g	Prejob Briefing Checklist complete (form 434.14)	Complete	7/17/01		

Check (Y) closed or assign an open item number

Review Team Leader: _____ Date:

UNIT D KING BUCK THERMAL CATALYTIC OXIDIZER PREFINAL INSPECTION CHECKLIST

	PROJECT TITLE				
ITEM NO.	ITEM DESCRIPTION	STATUS	DATE	PERSON RESPONSIBLE	COMMENTS
2	PROCEDURES AND WORK CONTROL DOCUMENTS				
a	List of chemicals used at VVET Unit D and required MSDSs are available.	Complete	7/17/01		
b	A Job Safety Analysis (JSA: RWMC-6572) has been completed for work activities. Hazard Checklist from MCP-3562. Hazard Evaluation Group walk down is complete.	Complete	7/17/01		
c	Emergency response plan is documented in the Health and Safety Plan (INEL-96/0119, Rev 5, May 2001).	Complete	7/17/01		In HASP
d	Hazardous Waste Determinations for identified waste streams are complete.	Complete	7/17/01		WDDF Form is ready
e	Points of contact and CERCLA signs are posted at VVET Unit D.	Complete	7/17/01		
f	Daily Inspection requirements have been established (Mechanical Inspection Round Sheet RS-045).	Complete	7/17/01		
g	Operating Procedure (TPR-1662) and Maintenance Work Orders (monthly (TRM-66), quarterly (TRM-67), semiannual (TRM-68), and annual (TRM-69)) approved and issued.	Complete	8/9/01	Lisa Harvego	Preventive maintenance work orders are complete
h	Observe startup / shutdown procedure (TPR-1662).	Complete	7/17/01		

Check (Y) closed or assign an open item number

Review Team Leader: _____ Date:

UNIT D KING BUCK THERMAL CATALYTIC OXIDIZER PREFINAL INSPECTION CHECKLIST

	PROJECT TITLE				
ITEM NO.	ITEM DESCRIPTION	STATUS	DATE	PERSON RESPONSIBLE	COMMENTS
3	PERSONNEL QUALIFICATION AND TRAINING				
a	All VVET Technicians have been identified and are available. Qualifications of the VVET Technicians should be documented.	Complete	7/17/01		Specific training in O&M Plan. HASP contains other training requirements.
b	VVET Technicians have been trained in the following as applicable:				
	OSHA 29 CFR 1910.120 (2002) 40-hour HAZWOPER	Complete	7/17/01		
	OSHA 29 CFR 1910.120 (2002) 8-hour supervisor (OS only)	Complete	7/17/01		
	First Aid/CPR	Complete	7/17/01		
	Radiological Worker II	Complete	7/17/01		
	Site Health and Safety Plan	Complete	7/17/01		
	Lock-out/Tag-out training (OS only)	Complete	7/17/01		
	Operations Procedures	Complete	7/17/01		
	Log keeping (MCP-2980 & MCP-231)	Complete	7/17/01		
	OCVZ Technician Training Plan	Complete	7/17/01		
	OCVZ Technician Qualification Checklist	Complete	7/17/01		

Check (Y) closed or assign an open item number

Review Team Leader: _____ Date:

UNIT D KING BUCK THERMAL CATALYTIC OXIDIZER PREFINAL INSPECTION CHECKLIST

	PROJECT TITLE				
ITEM NO.	ITEM DESCRIPTION	STATUS	DATE	PERSON RESPONSIBLE	COMMENTS
4	EQUIPMENT AND SYSTEM READINESS				
a	Subcontractor has completed checkout and component testing and deficiencies have been corrected. Shop test documentation in King Buck Technology O&M Manual Volume I and TPR-1764.	Complete	7/17/01		
b	Personal Protective Equipment is identified and available. PPE should be in the Health and Safety Plan (INEL-96/0119, Rev 5, May 2001).	Complete	7/17/01		Information is in HASP
c	**Medical and first aid supplies are identified and are available.** **This information should be in the Health and Safety Plan (INEL-96/0119, Rev 5, May 2001).**	Complete	7/17/01		Information is in HASP
d	Fire protection equipment is identified and available. Should be noted in the Health and Safety Plan (INEL-96/0119, Rev 5, May 2001).	Complete	7/17/01		Information is in HASP
e	Emergency communication equipment is identified and available. Requirement identified in the Health and Safety Plan (INEL-96/0119, Rev 5, May 2001).	Complete	7/17/01		Information is in HASP
f	Sampling equipment required to support sampling effort (e.g., summa canisters, field logbooks, and shipping containers) is identified and available Field Sampling Plan (INEEL/EXT-99-00907, Rev. 0, July 2000).	Complete	7/17/01		Information is in FSP
g	Sample analysis support services have been arranged.	Complete	7/17/01		
h	Freeze protection is in place.	Complete	7/17/01		Flex connection for frost heave

Check (Y) closed or assign an open item number

Review Team Leader: _____ Date:

UNIT D KING BUCK THERMAL CATALYTIC OXIDIZER PREFINAL INSPECTION CHECKLIST

	PROJECT TITLE				
ITEM NO.	ITEM DESCRIPTION	STATUS	DATE	PERSON RESPONSIBLE	COMMENTS
i	Recommended spare parts are available. A list of all required spares should be located in the O&M plan along with the location of the spares (INEEL/EXT-01-00016, Rev. B. July 2001).	Complete	7/17/01		
j	All components have been appropriately labeled. Check (isolation valves, air intake valves, and separator drain valve).	Complete	7/17/01		
k	Water level indicators and alarms on the vapor/liquid separator for warning and shutdown are functioning (TPR-1764).	Complete	7/17/01		
l	Check that manifold valve to well 7V is operational. Check that all lines and valves are numbered or named for respective wellhead locations.	Complete	7/17/01		
m	Mechanical inspection checklist is complete (King Buck Technology Shop Test documentation O&M Manual Vol. 1, TPR-1764, Daily Round Sheet RS-045).	Complete	7/17/01		
n	Daily Round Sheets (RS-045) available.	Complete	7/17/01		
o	Ensure SCADA system is operational (TPR-1764).	Complete	7/18/01		
p	Required calibrations and preventative maintenance have been identified and verified complete (O&M Plan (INEEL/EXT-01-00016, Rev. B. July 2001)).	Complete	7/17/01		
q	Modifications to the equipment from the original design have been documented (As built drawings available).	Complete	7/17/01		

Check (Y) closed or assign an open item number

Review Team Leader: _____ Date:

UNIT D KING BUCK THERMAL CATALYTIC OXIDIZER PREFINAL INSPECTION CHECKLIST

	PROJECT TITLE				
ITEM NO.	ITEM DESCRIPTION	STATUS	DATE	PERSON RESPONSIBLE	COMMENTS
5	OPERATION OF SAFETY SYSTEMS				
a	System shutdown Mechanisms have been satisfactorily tested (TPR-1764).	Complete	7/17/01		
b	**Administrative and operational limits have been established.** **Identify what the administrative and operational limits are and where they are located. (TPR-1662 warning and shutdown operational limits, logsheets operating specifications.)**	Complete	7/17/01		
c	Is radiation monitoring equipment in place and operational?	Complete	7/18/01		

Check (Y) closed or assign an open item number

Review Team Leader: _____ Date:

UNIT D KING BUCK THERMAL CATALYTIC OXIDIZER PREFINAL INSPECTION CHECKLIST

	PROJECT TITLE				
ITEM NO.	ITEM DESCRIPTION	STATUS	DATE	PERSON RESPONSIBLE	COMMENTS
6	MANAGEMENT PROGRAMS				
a	Personnel responsibilities and lines of authority are clearly defined (O&M Plan (INEEL/EXT-01-00016, Rev. B. July 2001)).	Complete	7/17/01		
b	Primary and Secondary emergency evacuation routes posted according to Health and Safety Plan (INEL-96/0119, Rev 5, May 2001) and RWMC access training.	Complete	7/17/01		
c	MANAGEMENT SELF-ASSESSMENT COMPLETED. Check documentation for completeness.	Complete	7/17/01		

Check (Y) closed or assign an open item number

Review Team Leader: _____ Date:

UNIT D KING BUCK THERMAL CATALYTIC OXIDIZER PREFINAL INSPECTION CHECKLIST

	PROJECT TITLE				
ITEM NO.	ITEM DESCRIPTION	STATUS	DATE	PERSON RESPONSIBLE	COMMENTS
7	ROUTINE AND EMERGENCY OPERATIONS PROGRAM				
a	System shutdown notification system is in place and has been tested for proper operation (RWMC back shift watch in accordance with RS-030)	Complete	7/17/01		
b	Security surveillance and notification requirements have been established with the facility security organization operation (RWMC back shift watch in accordance with RS-030)	Complete	7/17/01		

Check (Y) closed or assign an open item number

Review Team Leader: _____ Date:

UNIT D KING BUCK THERMAL CATALYTIC OXIDIZER PREFINAL INSPECTION CHECKLIST

	PROJECT TITLE				
ITEM NO.	ITEM DESCRIPTION	STATUS	DATE	PERSON RESPONSIBLE	COMMENTS
8	CONDUCT OF OPERATIONS				
a	Conduct of Operation Matrix is identified and available (LST-18)	Complete	7/17/01		
b	Appropriate logbooks are available	Complete	7/17/01		

***** END OF CHECKLIST *****

Check (Y) closed or assign an open item number

Review Team Leader: _____ Date: